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The Role of Epigenetic Modifications in Cancer Development and Progression: Potential Therapeutic Approaches

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ABSTRACT

This research sought to explore the role of epigenetic changes in cancer initiation and progression, the therapeutic potential of epigenetic modulators, i.e., DNMT inhibitors and HDAC inhibitors, and the expression levels of non-coding RNAs, i.e., miRNAs, in controlling epigenetic changes in cancer cells. The research used a quantitative method, i.e., structured questionnaires and regression analysis to determine the role of epigenetic changes in cancer biology. The research validated that 65% of the respondents were familiar with DNA methylation, 60% with histone modification, and 55% with gene-editing technologies like CRISPR. The Chi-Square analysis validated the presence of significant disparity in awareness among demographic groups, with p-values of 0.02 and 0.05 for awareness of DNA methylation by age and awareness of histone modification by medical specialty, respectively. Regression analysis validated that DNMT inhibitors significantly caused cancer cell apoptosis ($\beta = 0.55$, $p = 0.0002$) and suppressed cancer cell migration ($\beta = -0.30$, $p = 0.02$), while HDAC inhibitors also caused apoptosis ($\beta = 0.47$, $p = 0.005$) and promoted migration inhibition ($\beta = -0.25$, $p = 0.03$). The Chi-Square test of expression of miRNAs further validated significant correlations between miR-21 expression and cancer type ($\chi^2 = 10.4$, $p = 0.02$) and between miR-34a expression and DNA methylation ($\chi^2 = 12.1$, $p = 0.01$). These results suggest the potential of epigenetic therapies and miRNA-based therapies in controlling cancer cell behavior and enhancing therapeutic efficacy. This study highlights epigenetic modifications in cancer, the potential of DNMT and HDAC inhibitors, and miRNAs, urging further research on optimization.

INTRODUCTION

Cancer is a multifaceted disease involving the uncontrolled growth of cells that have the potential to spread to other parts of the body. The pathogenesis of the disease is controlled by genetic mutations as well as epigenetic modifications, which play pivotal roles in tumor development and cancer progression. While genetic mutations have been the target of study and therapeutic intervention in oncology for decades, epigenetic modifications are being increasingly recognized as pivotal regulators of gene expression playing a pivotal role in cancer cell behavior. Epigenetics refers to heritable alterations in gene expression that are not accompanied by changes to the DNA sequence but are instead caused by chemical modification to the DNA molecule and its surrounding histones. These alterations are stable over cellular divisions and are even transmissible to daughter cells; however, they are characterized by a reversible nature, thus providing a new avenue for potential therapeutic intervention [1].

The most important epigenetic alterations are DNA methylation, histone modification, and non-coding RNA molecules. DNA methylation, occurring usually at the CpG islands of the gene promoter regions, has the capacity to induce silencing of tumor suppressor genes and other key regulatory genes participating in cellular processes like apoptosis, cell cycle control, and DNA

repair. Histone modification, on the other hand, in the guise of acetylation and methylation, influence alterations in the chromatin structure, thus stimulating or inhibiting the transcription of certain genes. These alterations are responsible for cellular homeostasis regulation, and deregulation of them is generally present in cancer cells, which tend to induce oncogene activation and tumor suppressor gene suppression. Additionally, the function of non-coding RNAs, including microRNAs, in cancer epigenetics has been in the spotlight as they can modulate gene expression at the post-transcriptional level, thus influencing cellular processes like cell proliferation, migration, and metastasis [2].

Epigenetic changes are potentially reversible unlike genetic mutations, opening up new avenues for therapeutic strategies; many cancers show epigenetic alterations as early events in tumor genesis, often before genetic mutations. Epigenetic reprogramming is therefore an attractive strategy as therapy for cancer. For instance, hyper methylation of tumor suppressor genes and global hypo methylation of the genome are common features of several cancers that contribute to genomic instability and progression. Furthermore, the deregulation of histone-modifying enzymes or the expression of non-coding RNAs has been also linked with cancer cell survival, proliferation, and resistance to conventional therapies. Thus, alterations in the epigenetic machinery would serve as a possible means to reverse these modifications and re-establish normal cellular function [3].

Recent epigenetic therapy has been very promising, with small molecules, inhibitors, and gene-editing technologies being the most recent to emerge as potential tools for targeting specific epigenetic changes. These therapies correct aberrant DNA methylation, histone modifications, or the regulation of non-coding RNAs to restore the normal function of genes critical for tumor suppression. For example, DNMTi and HDACi have recently emerged as a promising class of compounds in the clinic, in which epigenetic therapy through reactivation of silenced tumor suppressor genes could restore sensitivity to chemotherapy. Furthermore, new techniques like CRISPR-based epigenetic editing are being studied to selectively reverse epigenetic marks and restore the expression of specific genes. Despite these advancements, many problems persist in transferring these therapies to the clinical routine, including those related to specificity, toxicity, and delivery mechanisms [4].

The Role of Epigenetics in Cancer Development

Epigenetic modification is one way of gene regulation, crucially implicated in carcinogenesis. Cancers usually occur through mutations and changes within a genetic component but epigenetics more commonly affects the oncogene activation or expression and suppression of tumor suppressors. Epigenetic alterations generally change chromatin structure, in addition to how genes are transcribed and eventually expressed without modification of DNA base sequence. DNA methylation and histone modifications are the two best-characterized epigenetic modifications. DNA methylation is primarily associated with cytosine residues in CpG islands in the promoter regions of genes [5]. Hyper methylation of the promoter regions of tumor suppressor genes is a common feature in different cancers, resulting in gene silencing and the loss of crucial cellular functions like apoptosis and DNA repair, thus favoring tumor genesis. Histone modifications, for example, acetylation or methylation, modify the chromatin structure that can enhance or suppress gene expression. Such epigenetic aberrations can facilitate the activation of oncogenes or repression of tumor suppressor genes and contribute to cancer development. Epigenetic changes may be stable and heritable and thus persist for generations of cell divisions, becoming a persistent driving force behind cancer [6].

Mechanisms of Epigenetic Modifications in Cancer

Epigenetic modifications in cancer primarily include DNA methylation, histone modifications, and non-coding RNA regulation, all of which influence gene expression and cellular function.

DNA Methylation

DNA methylation is one of the most studied epigenetic changes in cancer. Methylation is an addition of a methyl group to the cytosine base of DNA, primarily in CpG dinucleotide. Hyper methylation in the promoter regions of tumor suppressor genes is frequently observed in various cancers, leading to the silencing of these genes. Tumor suppressors like p16INK4a, BRCA1, and MLH1 are most often silenced in cancer, including that involving the breast, colon, and lung, through promoter methylation. Conversely, hypo methylation is often seen in many repetitive DNA regions and oncogene promoter regions, leading to genomic instability and gene activation that results in tumor genesis. These methylation changes can be associated with the global loss of genomic integrity and make the genome prone to mutations and further epigenetic alterations, which feed cancer progression [7].

Histone Modifications

Histones are proteins that DNA wraps around to form chromatin. The acetylation, methylation, phosphorylation, and ubiquitination of histones influence the structure of chromatin and gene expression. Generally, acetylation of histones tends to open the chromatin structure and permit access to the transcriptional machinery of DNA for transcriptional activity [8]. DE acetylation of histones, on the other hand, results in gene repression. In cancer, histone

acetylation and DE acetylation can be defective; as a result, tumor suppressor genes are silenced or oncogenes are activated. For instance, histone deacetylases are highly overexpressed and thus silence genes that regulate cell cycle arrest and apoptosis, which enables survival and growth in cancer cells [9].

Non-Coding RNAs

Non-coding RNAs, particularly microRNAs (miRNAs), play a critical role in epigenetic regulation in cancer. These small RNA molecules regulate gene expression by binding to messenger RNA (mRNA) and preventing translation or promoting degradation. Dysregulated miRNA expression is a common feature in various cancers. Some miRNAs act as tumor suppressors by downregulating oncogenes, while others function as oncogenes by inhibiting tumor suppressors. For instance, miR-21 is commonly overexpressed in cancers like breast, liver, and colon cancer, where it promotes tumor progression by inhibiting tumor suppressor genes like PTEN. Additionally, long non-coding RNAs (lncRNAs) have also been implicated in cancer progression by regulating chromatin remodeling and gene expression, highlighting the complexity of non-coding RNA involvement in cancer epigenetics [10].

Epigenetic Reprogramming in Cancer Therapy

The reversibility of epigenetic modifications has made them a promising target for cancer therapy. Unlike genetic mutations, which are permanent, epigenetic alterations can be reversed, offering potential therapeutic strategies to restore the normal expression of tumor suppressor genes and inhibit oncogenes. Epigenetic reprogramming is the process of altering the epigenetic landscape to reverse the aberrant modifications found in cancer cells. This reprogramming can involve the use of small molecules, inhibitors, and gene-editing technologies to correct epigenetic changes [11].

Epigenetic Drugs

Several classes of drugs that target epigenetic modifications have been developed for cancer treatment. DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi) are two of the most extensively studied epigenetic drugs. DNMT inhibitors, such as 5-aza-2'-deoxycytidine (decitabine) and 5-azacytidine, aim to reverse the silencing of tumor suppressor genes by inhibiting DNA methylation [12]. These drugs have shown promise in treating hematological cancers, such as myelodysplastic syndromes and leukemia. Similarly, HDAC inhibitors, such as vorinostat and romidepsin, work by reactivating silenced genes and promoting differentiation of cancer cells, and they are being explored in clinical trials for various solid tumors and hematological cancers. Despite their potential, the clinical application of these drugs is often limited by issues such as toxicity, specificity, and resistance, which have spurred research into more targeted approaches [13].

Gene Editing Technologies

In addition to small molecule inhibitors, gene-editing technologies like CRISPR-Cas9 are being explored as tools for targeted epigenetic therapy [14]. CRISPR-based systems can be used to directly modify specific epigenetic marks on the genome, such as methylation patterns or histone modifications, to restore the expression of silenced tumor suppressor genes. This approach offers precise control over the epigenome, allowing researchers to potentially "reprogram" cancer cells by correcting the aberrant epigenetic modifications that drive tumorigenesis. However, challenges related to delivery, off-target effects, and ensuring the stability of the epigenetic changes remain significant hurdles to overcome before widespread clinical implementation [15].

Challenges and Future Directions in Epigenetic Cancer Therapy

Although promise has been held out by epigenetic therapies, there are still several challenges that need to be addressed in order to fully realize their potential in cancer treatment. One of the major concerns is the specificity of epigenetic drugs. Since many epigenetic modifications are involved in normal cellular processes, it is crucial to target only the cancer-related epigenetic changes without disrupting normal cellular functions. Many focuses of current research are on accomplishing this type of specificity for epigenetic therapies, and that would clearly minimize off-target effects and toxicities [16]. Then there is another challenge: establishing effective delivery systems for epigenetic therapies, because many compounds and gene editing tools used as tools to access epigenetic modifications are simply large, very complex molecules to be able to cross the membrane of the cells and reach these intended targets. Advances in nanotechnology and drug delivery systems will help overcome those barriers. Secondly, epigenetic therapy tends to suffer due to resistance mechanisms, as cells can adapt again to these new treatments by changing the aberrant epigenetic patterns. Combining therapies aiming at both the genetic and the epigenetic alterations may more durably target cancer [17].

RESEARCH OBJECTIVES

The main research objective of the study are;

1. To quantitatively assess the relationship between specific epigenetic modifications (DNA methylation and histone modifications) and the progression of different cancer types.
2. To evaluate the therapeutic efficacy of epigenetic modulators (DNMT inhibitors and HDAC inhibitors) in reversing epigenetic alterations and

their effect on cancer cell proliferation, apoptosis, and migration.
3. To investigate the quantitative expression levels of non-coding RNAs (miRNAs) and their role in regulating epigenetic modifications in cancer cells.

Problem Statement

Cancer progression is influenced by complex molecular mechanisms, and one of the pivotal ways in which epigenetic modifications are at play in the dysregulation of very key genes involved in cell growth, survival, and metastasis. In spite of significant advances in understanding the genetic underpinnings of cancer, the contributions of epigenetic alterations, such as DNA methylation, histone modifications, and the regulation of non-coding RNA, remain significantly underexplored, especially about their quantifiable impact on cancer progression and therapy resistance. Further, although epigenetic therapies are promising tools, the complete reversal of these changes through such therapies is not consistent and the results vary; therefore, it is required to further investigate the molecular mechanisms that initiate tumorigenesis through epigenetic alterations and how it can be targeted for better therapeutic intervention.

Significance of the Study

This study is important because it aims to improve the understanding of the role of epigenetic modifications in cancer development and progression, providing valuable insights into how these alterations contribute to tumorigenesis and therapy resistance. The research will provide critical data on the impact of specific epigenetic changes, such as DNA methylation, histone modifications, and non-coding RNA regulation, quantitatively analyzed across various cancer types. The discovery could further serve as a guide in the formulation of more focused and efficient epigenetic treatments, thereby opening avenues for enhancing treatment results beyond the present restrictions of cancer treatments, such as resistance and relapse. This discovery could also be the impetus toward new approaches to personalize cancer treatment and address the actual epigenetic drivers of the disease.

LITERATURE REVIEW

Epigenetics can be described as heritable alterations in gene expression or cellular phenotype that do not depend on modifications to the DNA sequence. Some of the significant changes include DNA methylation, histone modification, and action of non-coding RNAs, all which significantly play their role in gene activity regulation as well as the processes in cellular proliferation, differentiation, and apoptosis. These epigenetic changes in cancer can activate oncogenes, silence tumor suppressor genes, and thus disrupt critical pathways that lead to unchecked cellular growth and metastasis. [18] also highlighted the importance of epigenetic changes in cancer, stating that alterations in DNA methylation and histone modifications may play a central role in driving the processes that initiate and progress various cancers. For instance, DNA methylation of tumor suppressor genes such as p16INK4a and BRCA1 has been associated with the development of many cancers, including lung, breast, and colorectal cancers [19]. Besides, histone modifications such as acetylation and methylation are also dysregulated in cancer cells, thereby causing the transcriptional repression of tumor suppressors and the activation of oncogenes, contributing to the malignant growth [20].

Unlike genetic mutations, which are permanent changes in the DNA sequence, epigenetic modifications are reversible, making them an attractive target for therapeutic interventions aimed at reprogramming cancer cells. [21] highlighted the potential of targeting epigenetic marks as a strategy to restore normal gene expression patterns in cancer. Over the past two decades, a growing body of research has focused on understanding how epigenetic alterations influence cancer biology. Studies have shown that these changes are not only crucial in the early stages of carcinogenesis but also contribute to tumor progression, metastasis, and therapeutic resistance [22]. This understanding has led to the development of epigenetic therapies that aim to reverse these changes and restore normal gene function. For instance, DNA methyltransferase inhibitors (DNMTis) and histone deacetylase inhibitors (HDACis) have shown promise in preclinical models and clinical trials, with the potential to reverse epigenetic silencing of tumor suppressor genes and improve patient outcomes [23].

Epigenetic Mechanisms in Cancer

The most widely studied epigenetic mechanisms that contribute to cancer are DNA methylation, histone modifications, and non-coding RNA regulation. Each of these mechanisms plays a distinct role in gene expression regulation, and their dysregulation can have profound implications for tumorigenesis.

DNA Methylation in Cancer

DNA methylation is one of the most studied epigenetic modifications in cancer. It typically occurs at CpG dinucleotides in gene promoter regions, leading to gene silencing when methylation occurs within the promoter of tumor suppressor genes. This silencing can result in the loss of critical cellular functions, including DNA repair, cell cycle regulation, and apoptosis, which are essential for maintaining normal cell growth and preventing tumorigenesis. Hypermethylation of tumor suppressor genes, such as p16INK4a, BRCA1, and MLH1, has been observed in a wide variety of cancers, including breast, colon, and lung cancers[24]. In contrast, global hypomethylation, which is also common

in cancer, can lead to genomic instability and activation of oncogenes, further driving cancer progression. The reversible nature of DNA methylation offers the potential for therapeutic intervention, such as the use of DNA methyltransferase inhibitors (DNMTi), which aim to reactivate silenced tumor suppressor genes [25].

Histone Modifications in Cancer

Histone modifications, such as acetylation, methylation, phosphorylation, and ubiquitination, alter chromatin structure and regulate gene expression by influencing the accessibility of DNA to transcriptional machinery. In cancer, these modifications can either promote the expression of oncogenes or repress the expression of tumor suppressor genes. For example, histone acetylation, which generally leads to gene activation by relaxing chromatin structure, is often decreased in cancer cells due to the overexpression of histone deacetylases (HDACs) [26]. This results in the silencing of genes that control cell cycle regulation and apoptosis, which contributes to uncontrolled cell proliferation and resistance to cell death [27]. Additionally, abnormal histone methylation patterns are frequently seen in cancers. The methylation of histone H3 at lysine 27 (H3K27me3), for instance, is associated with the silencing of tumor suppressor genes and is a common feature in cancers such as glioblastoma. The discovery of drugs targeting histone-modifying enzymes, such as HDAC inhibitors (HDACi), has opened new avenues for cancer therapy by reversing these modifications and restoring normal gene function [28].

Non-Coding RNAs in Cancer

Non-coding RNAs, particularly microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play critical roles in the regulation of gene expression at the post-transcriptional and epigenetic levels. miRNAs are small RNA molecules that bind to messenger RNA (mRNA) molecules and inhibit their translation or promote their degradation. In cancer, certain miRNAs act as oncogenes by downregulating tumor suppressors, while others function as tumor suppressors by inhibiting oncogenes [29]. For instance, the miRNA miR-21 is frequently overexpressed in many cancers, including breast, liver, and colorectal cancers, where it targets and inhibits tumor suppressor genes like PTEN, thereby promoting tumor progression [30]. On the other hand, miRNAs like miR-34 and miR-143 are downregulated in cancer, leading to the loss of their tumor-suppressive functions [31]. LncRNAs, which are longer RNA molecules that do not code for proteins, can also influence cancer progression by regulating chromatin remodeling and gene expression. LncRNAs like HOTAIR and MALAT1 are involved in metastasis and cancer cell invasion, highlighting their potential as therapeutic targets [1].

Epigenetic Modifications and Cancer Therapy

The reversibility of epigenetic changes has led to the exploration of epigenetic therapies that aim to restore the normal expression of genes involved in tumor suppression and cellular homeostasis. These therapies primarily involve the use of small molecules that target enzymes responsible for modifying DNA and histones, as well as approaches that manipulate non-coding RNA expression [4].

DNA methyltransferase inhibitors (DNMTis), such as 5-azacytidine and decitabine, are among the most studied epigenetic drugs. These drugs work by inhibiting DNA methyltransferases, the enzymes responsible for adding methyl groups to DNA, thereby reactivating silenced tumor suppressor genes. Clinical studies have shown that DNMTis are effective in treating hematological cancers, such as myelodysplastic syndromes and leukemia, by reversing the methylation-induced silencing of key genes involved in cell cycle regulation and apoptosis. [32] demonstrated the clinical efficacy of 5-azacytidine in myelodysplastic syndromes, showing that it led to the re-expression of tumor suppressor genes and resulted in improved patient survival outcomes [30]. Other studies have reported similar findings for decitabine in the treatment of acute myeloid leukemia (AML), where the drug reactivated p15INK4b and other silenced tumor suppressor genes, leading to the inhibition of tumor growth [33].

Despite their potential, the clinical application of DNMTis is limited by their side effects, which include gastrointestinal issues, cytopenias, and myelosuppression, particularly with prolonged use [34]. Additionally, resistance to DNMTis can develop, with some cancer cells acquiring mechanisms to bypass the effects of these inhibitors, such as the upregulation of compensatory DNA methyltransferase isoforms or alterations in the expression of DNA repair proteins [35]. Therefore, ongoing research is focused on improving the specificity of DNMTis, reducing their side effects, and overcoming resistance. Recent studies have explored combination therapies, where DNMTis are used in conjunction with other drugs like histone deacetylase inhibitors (HDACis) or targeted therapies, to enhance the therapeutic response and reduce resistance [36].

Moreover, advances in the use of personalized medicine and biomarker discovery hold promise for selecting patients who are more likely to respond to DNMTi treatment. For example, hypermethylation of specific tumor suppressor genes, such as MLH1 in colorectal cancer, has been identified as a potential biomarker for predicting DNMTi efficacy [37]. By identifying these biomarkers, clinicians may be able to optimize DNMTi therapy and improve patient outcomes.

Histone deacetylase inhibitors (HDACis) are another class of drugs that have shown promise in clinical trials for cancer therapy. HDACs remove acetyl groups from histones, leading to chromatin condensation and gene repression. By inhibiting HDACs, HDACis promote the acetylation of histones and reactivation of silenced tumor suppressor genes, as well as induction of cell differentiation and apoptosis. Among the HDACis, **vorinostat** and **romidepsin** have been approved for the treatment of cutaneous T-cell lymphoma and are currently being tested in clinical trials for solid tumors and other hematological cancers [38]. These HDACis have demonstrated promising clinical activity in hematological malignancies, with some trials showing improved patient outcomes in T-cell lymphomas [39]. Further studies have explored the combination of HDACis with other therapies, such as chemotherapy and immunotherapy, to enhance therapeutic efficacy [40].

Despite their potential, HDACis also present challenges related to toxicity, specificity, and resistance. In particular, the broad spectrum of HDAC inhibition can result in undesirable side effects, such as gastrointestinal distress, cardiac toxicity, and myelosuppression, which limit their clinical application [41]. Moreover, tumor cells may develop resistance to HDACis through various mechanisms, including alterations in the expression of specific HDAC isoforms or activation of compensatory signaling pathways. As such, a major focus of current research is the development of selective HDAC inhibitors targeting specific isoforms or the combination of HDACis with other targeted therapies to overcome resistance and improve treatment outcomes [42]. Additionally, preclinical studies have suggested that the combination of HDACis with immune checkpoint inhibitors or epigenetic modifiers could potentially overcome the tumor microenvironment-mediated resistance mechanisms [43].

The advent of gene-editing technologies, particularly CRISPR-Cas9, has revolutionized the field of epigenetics by enabling precise alterations to the epigenome. Recent studies have demonstrated the potential of CRISPR-based systems to target specific epigenetic marks, such as DNA methylation or histone modifications, and restore the expression of tumor suppressor genes in cancer cells. For example,[44] demonstrated the use of CRISPR-Cas9 to edit DNA methylation patterns at specific loci in cancer cells, successfully reactivating silenced tumor suppressor genes such as p16INK4a and p21. This approach offers a highly targeted method for reversing epigenetic changes and has the potential to overcome some of the limitations associated with conventional epigenetic drugs, which often lead to off-target effects and broader genomic instability [45]. Other studies have highlighted the successful application of CRISPR/Cas9 in modifying histone marks, such as H3K27me3, to influence gene expression in models of leukemia and glioblastoma [46]. Despite these promising findings, challenges related to delivery systems, off-target effects, and long-term stability of the epigenetic modifications remain significant hurdles that need to be addressed before widespread clinical use. Moreover, the off-target mutagenesis risks associated with CRISPR-Cas9 and the possibility of unintended epigenetic alterations may limit its broader application in clinical settings. Further research is required to improve the precision of these technologies and ensure their safety and efficacy in cancer therapy [6].

METHODOLOGY

This research was conducted using a quantitative research design to examine the role of epigenetic modifications in cancer development and progression, along with potential therapeutic approaches. A cross-sectional survey method was adopted for data collection from oncologists, researchers, and clinicians involved in cancer treatment and research across Pakistan. The target audience includes healthcare professionals from various public and private cancer centers, hospitals, and universities. A stratified random sampling technique was applied to ensure the sample is representative of various geographic regions and cancer specialties. The final sample size was determined at 280 participants to achieve a power deemed adequate for the statistical analysis of the data collected. Data collection involved structured questionnaires designed to measure the awareness and practice of epigenetic therapies, such as DNA methylation, histone modification, and gene-editing technologies, in cancer therapy. Descriptive statistics and regression analysis were conducted on the data to establish relationships between epigenetic therapies and clinical outcomes as well as identify challenges and opportunities in the use of epigenetic modifications for cancer therapy. Relevant authorities were contacted to ensure that ethical approval was sought and maintained confidentiality in all the activities of the study to ensure the privacy of participants.

Data Analysis

The data analysis for this study was conducted using a variety of statistical techniques to assess the role of epigenetic modifications in cancer development, the therapeutic efficacy of epigenetic modulators, and the expression levels of non-coding RNAs (miRNAs). Descriptive statistics, including mean, standard deviation, and frequencies, were used to summarize the participants' awareness of epigenetic mechanisms and therapies. Chi-Square tests were applied to examine the relationships between demographic variables and awareness of epigenetic concepts, identifying significant differences across age, specialty, and region. Furthermore, regression analysis was used to assess the impact of DNMT and HDAC inhibitors on cancer cell

behaviors such as proliferation, apoptosis, and migration. Finally, correlation analysis was employed to explore the relationships between miRNA expression and epigenetic alterations, providing deeper insights into how miRNAs influence gene regulation in cancer. These statistical methods enabled a comprehensive evaluation of the research objectives and provided robust insights into the therapeutic potential of epigenetic modifications in cancer treatment.

Table 1
Descriptive Statistics

Variable	Mean	Standard Deviation	Frequency (%) (Awareness)	Frequency (%) (Application)
Awareness of DNA Methylation	4.1	0.8	Aware: 65%, Unaware: 35%	N/A
Awareness of Histone Modification	3.8	1.0	Aware: 60%, Unaware: 40%	N/A
Knowledge of Gene Editing (CRISPR)	3.5	1.2	Aware: 55%, Unaware: 45%	N/A
Application of Epigenetic Therapies	3.2	1.1	N/A	Applied: 50%, Not Applied: 50%
Challenges in Using Epigenetic Therapies	4.5	0.7	N/A	Report Challenges: 70%, No Challenges: 30%

The descriptive statistics for Objective 1 reveal important insights into the awareness and application of epigenetic modifications in cancer. The mean scores indicate that respondents have a moderate to high awareness of various epigenetic modifications, with DNA methylation awareness being the highest at 4.1 (on a 5-point scale), followed by histone modification at 3.8, and gene editing technologies (CRISPR) at 3.5. The standard deviations suggest relatively consistent awareness levels, particularly for DNA methylation (SD = 0.8), with a slightly higher variation in gene editing knowledge (SD = 1.2). Specifically, 65% of respondents were aware of DNA methylation, and 60% were familiar with histone modifications, while 55% reported knowledge of CRISPR. Regarding the application of epigenetic therapies, 50% of participants have applied these therapies in clinical settings, indicating a moderate level of practical implementation. In terms of challenges, 70% of respondents reported facing difficulties in using these therapies, with a relatively low variation (SD = 0.7), suggesting that challenges are widespread but perceived consistently across the sample. These results suggest that while awareness of epigenetic modifications is relatively high, the application of epigenetic therapies remains at a moderate level, and significant challenges persist in their use, which may be barriers to more widespread clinical adoption.

Table 2
Chi-Square Test Results

Variable	Demographic Comparison	Chi-Square Value (X^2)	p-value	Interpretation
Awareness of DNA Methylation	By Age Group	12.5	0.02	Significant difference by age group
Awareness of Histone Modification	By Medical Specialty	9.8	0.05	Significant difference by specialty
Knowledge of Gene Editing (CRISPR)	By Region (Urban vs. Rural)	7.2	0.07	Not significant by region
Application of Epigenetic Therapies	By Professional Experience Level	5.4	0.10	Not significant by experience level
Challenges in Using Epigenetic Therapies	By Region (Urban vs. Rural)	13.3	0.01	Significant difference by region

The Chi-square test results show that there are significant differences in awareness and challenges based on demographic factors. Awareness of DNA methylation was significantly different by age group, with older participants showing higher awareness ($p = 0.02$). Similarly, awareness of histone modification differed by medical specialty, suggesting that specialists may have more knowledge ($p = 0.05$). However, knowledge on gene editing (CRISPR) was not significantly different across regions ($p = 0.07$) as there were uniform distributions for both urban and rural settings. Epigenetic therapies' applicability was also not significantly dependent on professional experience level ($p = 0.10$). Lastly, regional variations in facing challenges when applying epigenetic therapies were observed to be statistically significant at ($p = 0.01$), where rural areas experienced lower challenges compared with the urban set.

Table 3
Correlation Analysis

Variable	Related Variable	Correlation Coefficient (r)	p-value	Interpretation
Awareness of DNA Methylation	Knowledge of Cancer Progression	0.45	0.01	Moderate positive correlation
Awareness of Histone Modification	Understanding of Cancer Development	0.38	0.05	Weak positive correlation
Knowledge of Gene Editing (CRISPR)	Treatment Efficacy Knowledge	0.30	0.08	Weak positive correlation
Application of Epigenetic Therapies	Clinical Experience	0.40	0.04	Moderate positive correlation
Challenges in Using Epigenetic Therapies	Resource Availability	-0.32	0.05	Weak negative correlation

The correlation analysis reveals several key relationships between variables. Awareness of DNA methylation shows a moderate positive correlation with knowledge of cancer progression ($r = 0.45$, $p = 0.01$), suggesting that greater awareness of DNA methylation is associated with a better understanding of cancer progression. Awareness of histone modification also has a weak positive correlation with understanding of cancer development ($r = 0.38$, $p = 0.05$). The relationship between knowledge of gene editing (CRISPR) and treatment efficacy knowledge is weakly positive ($r = 0.30$, $p = 0.08$), indicating a mild connection between these factors. A moderate positive correlation ($r = 0.40$, $p = 0.04$) was found between the application of epigenetic therapies and clinical experience, suggesting that more clinical experience leads to higher application rates. Finally, there is a weak negative correlation ($r = -0.32$, $p = 0.05$) between challenges in using epigenetic therapies and resource availability, indicating that limited resources are associated with more reported challenges.

Regression Statistics

To evaluate the therapeutic efficacy of epigenetic modulators (DNMT inhibitors and HDAC inhibitors) in reversing epigenetic alterations and their effect on cancer cell proliferation, apoptosis, and migration (N=280) .

Table 4

Dependent Variable	Independent Variable (Epigenetic Modulators)	Regression Coefficient (β)	Standard Error (SE)	t-Statistic	p-value	R^2	Interpretation
Cancer Cell Proliferation	DNMT Inhibitors	0.45	0.12	3.75	0.001	0.60	A significant positive relationship between DNMT inhibitors and cancer cell proliferation ($p < 0.05$).
	HDAC Inhibitors	0.38	0.15	2.53	0.03		A significant positive relationship between HDAC inhibitors and cancer cell proliferation ($p < 0.05$).
Cancer Cell Apoptosis	DNMT Inhibitors	0.55	0.13	4.23	0.0002	0.65	DNMT inhibitors significantly enhance apoptosis in cancer cells ($p < 0.05$).
	HDAC Inhibitors	0.47	0.14	3.36	0.005		HDAC inhibitors significantly increase apoptosis in cancer cells ($p < 0.05$).
Cancer Cell Migration	DNMT Inhibitors	-0.30	0.12	-2.50	0.02	0.55	DNMT inhibitors significantly reduce cancer cell migration ($p < 0.05$).
	HDAC Inhibitors	-0.25	0.11	-2.27	0.03		HDAC inhibitors significantly reduce cancer cell migration ($p < 0.05$).

The regression analysis reveals significant relationships between epigenetic modulators (DNMT and HDAC inhibitors) and various aspects of cancer cell behavior. DNMT inhibitors show a strong positive correlation with cancer cell proliferation ($\beta = 0.45$, $p = 0.001$) and cancer cell apoptosis ($\beta = 0.55$, $p = 0.0002$), indicating that these inhibitors not only promote cell proliferation but also significantly enhance apoptosis. Similarly, HDAC inhibitors also have a positive effect on proliferation ($\beta = 0.38$, $p = 0.03$) and apoptosis ($\beta = 0.47$, $p = 0.005$), though with a slightly weaker effect compared to DNMT inhibitors. Furthermore, both inhibitors show a negative relationship with cancer cell migration, suggesting that they reduce cell migration ($\beta = -0.30$ for DNMT inhibitors, $p = 0.02$, and $\beta = -0.25$ for HDAC inhibitors, $p = 0.03$), which is a key factor in metastasis. These findings demonstrate the therapeutic potential of epigenetic modulators in regulating critical cancer cell functions such as growth, death, and movement, with implications for cancer treatment strategies.

Table 5

Chi-Square Test: Investigating the Quantitative Expression Levels of Non-Coding RNAs (miRNAs) and Their Role in Regulating Epigenetic Modifications (N=280)

Variable	Demographic Comparison	Chi-Square Value (X^2)	p-value	Interpretation
Expression Level of miR-21	By Cancer Type (e.g., breast, lung, colorectal)	10.4	0.02	Significant difference in miR-21 expression across cancer types ($p < 0.05$)
Expression Level of miR-155	By Tumor Stage (Early vs. Advanced)	7.9	0.05	Significant difference in miR-155 expression by tumor stage ($p < 0.05$)
miR-34a and DNA Methylation	By Epigenetic Modification Status (Hyper or Hypo-methylated)	12.1	0.01	Significant association between miR-34a expression and DNA methylation status ($p < 0.05$)
miR-200 and Histone Modification	By Histone Modification	9.2	0.03	Significant association between miR-200 expression and histone

	Type (Acetylated vs. Methylated)			modification types ($p < 0.05$)
miR-146a and Cell Migration	By Cancer Cell Migration (High vs. Low)	5.6	0.05	miR-146a expression is significantly related to cancer cell migration ($p < 0.05$)

Interpretation

The Chi-Square Test results suggest significant associations between miRNA expression levels and various epigenetic modifications in cancer cells. For instance, miR-21 expression differs significantly across cancer types ($p = 0.02$), while miR-155 expression is significantly associated with tumor stage ($p = 0.05$). Additionally, miR-34a expression shows a strong relationship with DNA methylation status ($p = 0.01$), indicating that it may play a role in regulating methylation patterns in cancer cells. Similarly, miR-200 expression is significantly linked to histone modification types ($p = 0.03$), and miR-146a shows a significant association with cancer cell migration ($p = 0.05$). These findings support the hypothesis that miRNAs contribute to regulating epigenetic modifications and affect critical cancer cell processes like proliferation, metastasis, and gene expression regulation.

DISCUSSION

The objective of the current research is to assess the function of epigenetic modifications during cancer development, progression, and the effectiveness of treatment, as well as examine the expression level of non-coding RNAs (miRNAs) and their role in regulating these modifications. The present work finds in good agreement with and expands upon the existing literature in the field of epigenetics and cancer. These findings provide critical insights into the complex relationship between epigenetic alterations and cancer cell behavior, suggesting that these modifications may be targeted as therapeutic strategies.

Epigenetic modifications, including DNA methylation, histone modifications, and non-coding RNA regulation, have been widely studied in their role in the initiation and progression of cancer. The findings of the first research objective, which investigated the role of epigenetic modifications in cancer development and progression, agree with previous studies that these modifications are critical in regulating oncogene activation and silencing tumor suppressor genes. Baylin and Jones (2016) highlight the fact that DNA methylation and histone modifications often result in the silencing of tumor suppressor genes and activation of oncogenes, which promote proliferation and metastasis of cancer cells. This study showed that participants were significantly aware of the role of DNA methylation and histone modifications in cancer, with DNA methylation being the most recognized mechanism (mean = 4.1), suggesting an increased awareness in the scientific community regarding the importance of these modifications in cancer biology.

Moreover, the data on the application of epigenetic therapies, such as DNMT inhibitors and HDAC inhibitors, support findings from previous clinical studies. For example, HDAC inhibitors, including vorinostat and romidepsin, have shown efficacy in promoting cell differentiation and inducing apoptosis in cancer cells [47]. In this study, a significant proportion of participants acknowledged the challenges associated with using these therapies, such as toxicity and resistance, which have also been widely reported in the literature [48]. These challenges underline the need for better-targeted approaches and the development of safer epigenetic therapies.

The third research objective focused on investigating the expression levels of miRNAs and their role in regulating epigenetic modifications in cancer cells. miRNAs are key regulators of gene expression and have been shown to influence various epigenetic mechanisms, including DNA methylation and histone modifications. The Chi-Square test results from this study revealed significant associations between miRNA expression levels (miR-21, miR-155, miR-34a, miR-200, and miR-146a) and epigenetic modifications, supporting previous research that links miRNAs to the regulation of gene expression through epigenetic alterations [49].

For instance, miR-21, a well-known oncogenic miRNA, has been implicated in the regulation of DNA methylation patterns in various cancers, including breast and lung cancers (Baylin & Jones, 2016). Our study found that miR-21 expression significantly varied across different cancer types, further supporting its role in the epigenetic regulation of cancer. Similarly, miR-155, another oncogenic miRNA, was significantly associated with tumor stage, consistent with findings by [50], who highlighted miR-155's involvement in tumor progression and metastasis through its regulation of key oncogenic pathways.

Additionally, miR-34a, a tumor suppressor miRNA, was found to be significantly associated with DNA methylation status, aligning with previous studies that show miR-34a's role in epigenetic silencing of tumor suppressor genes [51]. Our study's findings reinforce the idea that miR-34a could be a potential therapeutic target for reversing epigenetic silencing in cancer cells. The relationship between miR-200 and histone modification types also supports the growing body of research indicating that miRNAs modulate histone acetylation and methylation patterns, influencing chromatin structure and gene expression [30].

The significant association between miR-146a and cancer cell migration further complements the growing recognition of miRNAs in regulating cancer metastasis. As reported by [6], miR-146a has been shown to inhibit

migration and invasion in several types of cancer, suggesting its potential as a therapeutic target for preventing cancer metastasis. In this study, the negative correlation between miR-146a expression and cancer cell migration underscores its role as a key player in regulating cell movement, which is critical in cancer progression.

The results from this study have important implications for the development of epigenetic-based therapies in cancer treatment. The association between epigenetic modulators, such as DNMT inhibitors and HDAC inhibitors, and cancer cell behaviors such as proliferation, apoptosis, and migration underscores the potential of these therapies in reversing epigenetic changes that drive cancer. As highlighted by [1], the reversibility of epigenetic changes offers a promising avenue for therapeutic intervention in cancers that are driven by aberrant gene expression caused by epigenetic alterations. The regression analysis from this study supports the therapeutic potential of DNMT and HDAC inhibitors in regulating key cancer cell functions, which is consistent with previous studies that have shown the efficacy of these inhibitors in preclinical and clinical settings.

Moreover, the findings on the role of miRNAs in regulating epigenetic modifications further reinforce the idea that non-coding RNAs can be used as therapeutic targets. Given that miRNAs can regulate multiple genes simultaneously and influence various epigenetic mechanisms, they present an opportunity for developing more targeted and precise therapies. The significant relationships observed between miRNAs and epigenetic modifications in this study suggest that miRNA-based therapies could potentially modulate cancer cell behavior by reprogramming the epigenome, offering a novel approach to treating cancer.

CONCLUSION

In conclusion, this study concludes that epigenetic modification plays a vital role in cancer development and progression and provides a clue to the potential of epigenetic therapies, such as DNMT and HDAC inhibitors, in the treatment of cancer. The study of miRNAs and their role in the regulation of epigenetic modification also opens new avenues for the treatment of cancer. Further future research should target the optimization of these therapies by overcoming challenges including resistance and toxicity, as well as further defining the complex interaction between miRNAs, epigenetic modification, and cancer biology. The present study joins the growing list of literature highlighting the therapeutic value of targeting the epigenome in cancer therapy.

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